A. Interview with Examiners Wortman and Rawlings on August 7, 2002

Applicants and their attorneys thank Examiner Wortman and Examiner Rawlings for the courtesies extended during the interview on August 7, 2002. In response to the Examiners' request at the August 7, 2002 interview, applicants have prepared this response to supplement the response submitted on March 8, 2002.

II. The rejection of record relating to the Cabib et al. reference

Cabib et al. is relied on in the Action mailed November 26, 2001 for teaching spectral imaging methods for *in situ* medical diagnosis and treatment comprising preparing a sample to be imaged, viewing the sample through an optical device optically connected to a spectrometer, collecting and measuring incident light using a detector and collecting and interpreting data using a mathematical algorithm. It is asserted in the Action mailed November 26, 2001 that 1) "numerous examples of *in situ* analyses of cells and/or tissues to either classify and/or diagnose cellular abnormalities in said cells and/or tissues are provided" in Cabib et al., and Examples 1, 6, 7 and 8 are pointed to with particularity; 2) Cabib et al. "discloses that a metachromatic dye, such as Azure-B, which is a thiazine dye, can be used to practice the prior art methods (see Example 2, column 43, line 10)"; 3) "the sample of tissue or cells to be analyzed [in Cabib et al.] is prepared by staining with either Romanowsky-Giemsa stain, haematoxylin-eosin stain, or May-Grunwald-Giemsa stain (see claim 59), each of which are compositions comprising thiazine dyes"; 4) Cabib et al. teaches "that a spectral component may 'correlate well with what is called the purple Romanowsky-Giemsa complex'"; and 5) "an objective of the prior art invention is to distinguish cancer from healthy or otherwise diseased tissue or cells (column 6, lines 27-33)." It is further asserted in the Interview Summary mailed July 22, 2002 that "the method taught by the prior art is deemed inherently the same as the method of the claims despite the fact that the prior art does not make use of the term 'metachromatic shift' to describe the spectral differences between a test sample and the library of spectra." Applicants respectfully traverse this rejection. Applicants will first briefly describe the claimed invention and then explain why Cabib et al. does not teach or suggest, either expressly or inherently, such an

invention. In light of the discussion below, it is believed that the above objection and rejections of record have been overcome and that all pending claims are in condition for allowance. Action toward this end is respectfully solicited.

III. The Invention

The present invention relates to a method for diagnosing a disease state *in situ* in biological tissue or cells of a living organism that includes utilization of spectroscopic methods to analyze the metachromatic properties of various dyes in abnormal (e.g., dysplastic, pre-cancerous and cancerous) and normal cells. The inventors of the present invention have surprisingly discovered that the extent of the metachromatic shift observed in a dye from stained tissue or cells can be used to differentiate, for example, the aforementioned abnormal cells and/or tissues from normal cells and/or tissues. According to one embodiment of the methods described in the application, the metachromatic shift of a dye observed in the reflected light spectrum from a dye-stained test sample is compared to the metachromatic shift of the dye from a library of previously obtained spectra of similarly stained tissue or cells wherein the diagnosis of the disease state of the reference cells or tissue upon which the previously obtained spectra are based was confirmed by conventional histochemical methods. Thus, quicker, more precise diagnoses may be made according to the present method compared to existing methods.

IV. The Cabib et al. reference

Cabib et al. do not expressly or inherently teach or suggest quantifying the extent of metachromatic shift of a metachromatic dye in a stained test sample and making a correlation with a dysplastic, pre-cancer or cancer disease state using this information as recited in the pending claims. The following three points will be further discussed and support this assertion: (1) When the Cabib et al. reference is viewed as a whole, it is seen to primarily relate to detecting spatial organization and quantifying cellular and tissue natural constituents and does not teach or suggest methods of diagnosing dysplasia, pre-cancer or cancer by quantifying, for example, the metachromatic shift of

a metachromatic dye; (2) Cabib et al. teach use of dyes as contrast agents to visualize structures (e.g., to look at biological components and/or their spectrum) and do not teach or suggest, either expressly or inherently, use of metachromasia for any diagnostic purpose; (3) one skilled in the art would not recognize that metachromasia could be used in a diagnosis of dysplasia, pre-cancer or cancer from the teaching of Cabib et al.

A. Cabib et al. relate to detecting spatial organization and quantifying cellular and tissue natural constituents and any method of diagnosing cancer discussed in Cabib et al. involves use of such information, not information relating to quantifying the metachromatic shift of a metachromatic dye

1. Cabib et al. teach detecting spatial organization and quantifying cellular and tissue natural constituents

It is clear that, throughout the patent, Cabib et al. teach that the methods disclosed therein are used to detect and quantify cellular and tissue constituents. For example, column 1, lines 17-21, provides that "[t]he methods of the present invention can be used to detect spatial organization (i.e., distribution) and to quantify cellular and tissue natural constituents, structures, organelles and administered components such as tagging probes (e.g., fluorescent probes) and drugs...." It is further seen in column 6, lines 36-39, of Cabib et al. that "[t]he method further enables the identification and spatial mapping of proteins, [sic] sacharides, [sic] AND+ and NADH, collagen, elastin and flavin, and various additional metabolic mediators within cells and/or tissues." Additionally, it is stated in column 6, lines 27-33, that "[a]nother objective of the present invention is to map in a quantitative way white light, ultraviolet or laser-induced emission spectra from biological components (e.g., oxygenated and deoxygenated hemoglobin in retinal blood vessels and or melanin pigmentation level in the retina) and, to distinguish cancer from healthy, or otherwise diseased tissue or cells." It will be appreciated from this statement that Cabib et al. are relying on the spectra of the biological components in making a determination of cancer. This conclusion is buttressed by the methods discussed in Cabib et al. to map cancerous tissue as described in Example 7.

In the only example of mapping cancerous tissue *in vivo* (Example 7), which is a prophetic example, in colon, bladder, lungs, cervix and other internal organs, it is mentioned that the procedure is similar to the ophthalmologic examples in Example 6. It is mentioned, in column 58, lines 40-44, that the differences between the method of Example 7 and Example 6 is in the collecting optics utilized, in the types of some basic molecular components involved in the detection: "some of these are probably common, such as oxygen concentration, additional others are collagen and elastin, genetic material in the cell nuclei, such as DNA chromatin, etc." Example 6 involves diagnosis of retinal abnormalities by measuring oxygen concentrations. However, it is also mentioned in column 56, lines 58-61, of Example 6 that, besides measuring oxygen by measuring the concentration of hemoglobin, "important information can be obtained also by measuring the concentration of other constituents, such as NAD^+, NADH, flavin, cytochromes, etc." No dyes are taught or suggested to be used in this example. Cabib et al. either rely on the inherent spectra of biological components, as discussed above, or the use of dyes as contrast agents as discussed below.

2. Cabib et al. teach use of dyes as contrast agents to visualize cellular structures

Any mention of use of dyes in Cabib et al. relates to their use as contrast agents. At column 11, lines 24-28, it is mentioned that a sample is stained using a method selected from the group consisting of Romanowsky-Giemsa staining, Haematoxylin-Eosin staining and May-Grunwald-Giemsa staining. Additionally, for example, in column 36, lines 17-22, it is mentioned that "[t]ransmission microscopy suffers greatly from the inherently low contrast of cell organelles and structural details. *Many methods have been developed to improve this contrast, among them staining* and spatial filtering." (Emphasis added). Cabib et al. go on to discuss staining techniques to facilitate histological examination using organic stains which specifically bind to different macromolecules in cells and mention that the most common staining techniques are Romanowsky-Giemsa stain (eosin Y and azure B), and Haematoxylin-Eosin. Cabib et al. further mention, in column 37, lines 9-12, that "[w]hatever the technique, *with staining it is possible to distinguish between subcellular compartments of the cell and especially*

to distinguish the chromatin organization in the nucleus." (Emphasis added). The Romanowksy-Giemsa complex that Cabib et al. mention *may* form when staining with the Romanowsky-Giemsa stain is described only in fixed tissue.

Additionally, Example 2, involving the measurement of the ratio of heterochromatin to euchromatin (i.e., cellular structures) in stained tissue to study chromatin condensation further underscores the use by Cabib et al. of dyes as contrast agents to visualize cellular structures. For example, Cabib et al. mention in column 38, line 66 to column 39, line 9, that "[t]he spectral cytoplasmic features (spectrum B of FIG. 9a), when used for similarity mapping, allow the clear demarcation of components which one believe [sic] represent the nuclear envelope, Golgi cisternae, cytoplasmic vacuoles, and the outer cell membrane." Moreover, column 43, line 10, of Example 2 referred to in the Action discloses that "[s]tandard analysis of blood cells is based on staining with either May-Grunwald-Giemsa or Romanowsky techniques which employ the dyes azure-B and Eosin." Such blood cell analysis involves examination of various cellular structures. For example, analysis of bone marrow cells, precursors to red blood cells, in Example 2 includes quantitation of euchromatin and heterochromatin and morphological analysis of cytoplasmic components and does not involve metachromasia.

Example 8 of Cabib et al. referred to in the Action teaches staining a cervical smear with haematoxylin-eosin for aiding in the diagnostic pathology as analyzed by a transmission microscopy RGB image. Haematoxylin-eosin is one of the most commonly used stains in histopathology, and does not include a metachromatic dye. Haematoxylin is a basic dye that stains acidic structures (e.g., DNA, ribosomes and rough endoplasmic reticulum) a purplish-blue. Eosin is an acidic dye that stains basic structures red or pink (e.g., it stains basic cytoplasmic proteins pink or pinkish-red). The stain is clearly used in this example as a contrast agent in order to quantitate various cellular structures to aid in the diagnosis. This staining procedure is commonly performed in the art and is not relevant to applicants claimed invention.

For example, applicants have claimed a method for diagnosing dysplasia, pre-cancer or cancer *in situ* in biological tissues or cells of a living organism. *In situ* refers both to *in vivo* and *ex vivo* (i.e., freshly excised and otherwise living tissue). Prior to microscopically examining a Pap smear in the prior art, the sample is mounted on a

slide, fixed and then stained. Such fixing significantly alters the underlying biochemistry of the cells/tissue such that the interaction of the stains is entirely different than if the stains are applied *in situ* to either freshly excised tissue or to a surgical site directly on the patient. The transverse thin sections are orthogonal to the cell membranes, leaving little of the membrane presented for viewing other than the edge. Conversely, the unique correlation with the metachromasia of the thiazine dyes with the disease state of the tissue as discovered by the inventors of the present invention relies on use of intact cells having an intact cell membrane. The unique sensitivity specifically of methylene blue and toluidine blue O, and thiazine dyes generally, for abnormal cells, such as pre-cancerous and cancerous cells, is due to the unique properties of the dyes (e.g., lipophilic, cationic, low molecular weight) which are readily taken up by the significantly modified cell membrane of such cells. For example, the enhanced membrane permeability of pre-cancerous and cancerous cells for these compounds appears to be in excess of the chemical activity needed for transport across a gradient potential. A fixed thin section neither has an active cell membrane potential to exclude or include the dye in an active manner, nor sufficient cell membrane presented to the viewer to determine the extent of dye localization.

Although Cabib et al. discuss use of stains in the methods described therein as contrast agents, it is discussed therein that is it preferable not to use dyes at all, thus adding support that Cabib et al. do not teach or suggest that the dyes discussed therein have diagnostic value as found by the inventors of the present invention. For example, it is mentioned in column 39, line 13-16 of Cabib et al. that "[o]ne of the advantages of combining spectral bio-imaging and transmission microscopy is the ability to use a 'clean' measurement technique, i.e., no need for working with potentially toxic dyes or fixation agents." By contrast, the present inventors have recognized the diagnostic value of the dyes when employed according to the invention, and have taught the art how to extract, interpret and utilize this heretofore unappreciated information to advance human health.

B. One skilled in the art would not recognize that metachromasia could be used in a diagnosis of dysplasia, pre-cancer or cancer from the teachings of Cabib et al.

Turning now to the assertion in the Interview Summary mailed July 22, 2002 that "the method taught by the prior art is deemed inherently the same as the method of the claims despite the fact that the prior art does not make use of the term 'metachromatic shift' to describe the spectral differences between the test sample and the library of spectra which contain positive and negative controls", please consider that, to establish inherency, the extrinsic evidence "'must make clear that the missing descriptive matter is necessarily present in the thing described in the reference, and that it would be so recognized by persons of ordinary skill." *In re Roberston*, 49 U.S.P.Q.2d 1949, 1950-1951 (Fed. Cir. 1999) (citations omitted). "Inherency, however, may not be established by probabilities or possibilities. The mere fact that a given thing may result from a given set of circumstances is not sufficient.'" *Id.* (citations omitted). "In relying upon the theory of inherency, the examiner must provide a basis in fact and/or technical reasoning to reasonably support the determination that the allegedly inherent characteristic *necessarily* flows from the teachings of the applied prior art." *Ex parte Levy*, 17 U.S.P.Q.2d 1461, 1464 (Bd. Pat. App. & Inter. 1990) (emphasis in original). With respect, it is submitted that the Examiner has not met this burden for, *inter alia*, the following reasons.

There is no mention of toluidine blue O in the Cabib et al. Cabib et al. teach use of a stain composition that includes two dyes – eosin Y and either methylene blue (as in May-Grunwald-Giemsa) or azure B (as in Romanowsky-Giemsa). There is no teaching in Cabib et al. from which the person of ordinary skill could conclude that the method of Cabib et al., employing a combination of eosin Y and either methylene blue or azure B, inherently, that is, necessarily, utilizes a metachromatic shift to diagnose dysplasia, pre-cancer or cancer, because, *inter alia*, the dye compositions of Cabib et al. employ a non-metachromatic dye, and Cabib et al. teach a method based on contrast, not metachromasia. Contrary to the Examiner's assertion, the use of positive and negative controls for the contrast dye compositions of Cabib et al. does not convert the teaching of that reference into applicants' claimed invention, and neither would one of ordinary

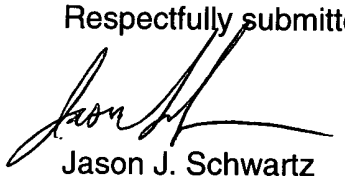
skill in the art conclude from Cabib et al. that a metachromatic shift is necessarily and inherently taking place. In fact, it is only by the improper use of applicants' teaching that the Examiner can supply, in hindsight, the deficiencies of Cabib et al.

V. Conclusion

Cabib et al. do not teach or suggest, either expressly or inherently, the method as recited in the claims of the pending application. Withdrawal of the rejection of claims 1, 5 and 7-11 under 35 U.S.C. § 102(b) or 102(e) is respectfully requested.

In light of the foregoing discussion, it is believed that claims 1, 5 and 7-11 are in condition for immediate allowance. Action towards this end is respectfully requested. The Examiner is invited to telephone the undersigned attorney regarding any issues that may be handled in that fashion.

Respectfully submitted,



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